S4.29 Catalytic properties of Na^+-translocating NADH:quinone oxidoreductases from *Vibrio harveyi*, *Klebsiella pneumoniae*, and *Azotobacter vinelandii*

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The main goal of our study was a comparative analysis of the catalytic properties of sodium-translocating NADH:quinone oxidoreductases (Na^+-NQRs) from marine bacterium *Vibrio harveyi*, enterobacterium *Klebsiella pneumoniae*, and soil microorganism *Azotobacter vinelandii*. It is shown that their enzymes drastically differ in their affinity to sodium ions with apparent K_M values 2.7 mM, 0.67 mM and ≈0.1 mM respectively. The enzymes also possess different sensitivity to inhibitors. Na^+-NQR from *A. vinelandii* is not sensitive to low HQNO concentrations, while Na^+-NQRs from *V. harveyi* and *K. pneumoniae* can be inhibited with $I_{0.5}$ values 0.13 μM and 0.55 μM respectively. Also Na^+-NQR from *K. pneumoniae* is fully resistant to either Ag^+ (which is considered to be specific inhibitor of Na^+-NQR from *V. harveyi*) or *N*-ethylmaleimide. Na^+-NQR from *A. vinelandii* possess transitional sensitivity to these modificators of SH-groups. All the Na^+-NQR-type enzymes are sensitive to diphenyliodonium. So the main unique characteristic of Na^+-NQR is its specific requirement for sodium ions, which can be not readily detectable, since the affinity of Na^+-NQR to Na^+ can be very high.

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(S5) Mitochondrial biogenesis symposium lecture abstracts

S5/1 Control of the synthesis of uncoupling and coupling proteins in brown adipose tissue

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In active brown adipose tissue, the balance between coupled and uncoupled respiration is the opposite of that seen elsewhere. This is accomplished through two features: low content of coupling proteins (the ATP-synthase complex) and high content of uncoupling protein (UCP1). In the tissue, very high expression (mRNA levels) of all subunits of ATP-synthase is seen — except for subunit c, implying that ATP-synthase assembly is under control of sub-c amount. We have now demonstrated that artificially-induced overexpression of sub-c results in increased amounts of fully competent ATP-synthase. In wildtype, despite high mRNA levels for the other subunits, no unassembled ATP-synthase subunits are observed in blue-native gels. This implies translation control of the other components of the ATP-synthase. Concerning the β-subunit, the control in different tissues may be related to formation of an RNA/protein complex that is dependent on a stem–loop structure in the 3′UTR mRNA. Brown adipose tissue recruitment and UCP1expression are normally considered to be under sympathetic control. There is physiological reason for a nonsympathetic recruitment pathway. We find that chronic treatment of brown (pre)adipocytes with PPARγ-agonists activates mitochondriogenesis and UCP1 expression, leading to thermogenically competent brown-fat cells, i.e. cells that although naive to norepinephrine respond thermogenically to norepinephrine.

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S5/2 Regulation of mitochondrial dynamics by nitric oxide is a key event in myogenesis

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Mitochondria exist in two interconverting forms, i.e. as small isolated particles, and as extended filaments, networks or clusters. Here we provide evidence that in differentiating myoblasts endogenous nitric oxide (NO) generation controls mitochondrial shape: in the absence of NO mitochondrial fission occurs rapidly. The action of NO is specifically addressed to mitochondrial fission since in PEG fusion assay organelle fusion was not modified by the treatment with the NO synthase-inhibitor L-NAME. A key protein involved in mitochondrial fission is the large GTPase DRP-1. DRP-1 translocation to the mitochondria promotes mitochondrial fission. DRP-1 translocation and mitochondrial fission were stimulated by L-NAME and inhibited by exogenous NO. In addition, NO inhibited DRP-1 GTPase activity. We also found that in differentiating myoblasts NO is required for the expression of differentiation markers including myogenin and muscle specific myosin since L-NAME inhibited myogenic differentiation, and exogenous NO restored it. Overexpression of a dominant negative DRP-1 reversed the inhibitory effect of L-NAME on myogenesis. Our results indicate that NO controls a key event in mitochondrial dynamics that may have relevant implications for both myogenesis and control of energy metabolism in developing skeletal muscle.

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S5/3 Bioenergetics of mitochondrial protein topogenesis

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The TIM23 translocase is involved in the topogenesis of the vast majority of mitochondrial proteins. Preproteins pass through the TOM complex of the outer membrane and are then transferred across or laterally inserted into the inner membrane (IM). The electrical membrane potential $\Delta\psi$ is required for the translocation of the targeting signal across the IM where it can be reached by the chaperone mtHsp70. Further translocation does not require $\Delta\psi$, but instead matrix ATP. ATP hydrolysis drives cycles of binding of mtsp70 to incoming unfolded preproteins. mtHsp70 is part of the mitochondrial import motor which comprises further components,